PATENT
P-4739-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Moutsatsos et al.	Examiner:	Sandals W.
Serial No.:	09/148,234	Group Art Unit:	1636
Filed:	September 4, 1998		
Title:	GENETICALLY ENGINEERED CELLS WHICH EXPRESS BONE MORPHOGENIC PROTEINS		

DECLARATION UNDER RULE 37 C.F.R. 1.132

Assistant Commissioner for Patents
Washington, DC 20231

I, Edward Schwarz, Ph.D., a citizen of the United States of America, residing at 125 Barclay Square Drive, Rochester, New York, 14618, USA, hereby declare:

1. I am a Professor of Orthopedics, Medicine, Biomedical Engineering, Pathology, Microbiology and Immunology at the University of Rochester, Rochester, New York, USA. I have a Ph.D. in Microbiology and Immunology from the Sue Golding Graduate Division of the Albert Einstein College of Medicine, Bronx, New York. My fields of expertise are Molecular Biology, Gene Therapy, Stem Cell Biology and Musculoskeletal Tissue Engineering. Specifically, I have been involved in the study of revitalizing bone grafts using gene therapy and stem cell approaches.

2. My Curriculum Vitae and list of publications are attached herewith as Appendix 1.

3. I have read the subject Application and have reviewed the patent Prosecution History, including the Office Action of April 04, 2006, November 15, 2005, July 21, 2005, December 7, 2004, October 22, 2004, June 21, 2004, and September 9, 2003. The subject Application describes *inter alia*, *ex-vivo* methods of transforming or transducing mesenchymal stem cells with a nucleic acid, which encodes for BMP-2 protein, for implantation in a subject in need of bone repair or regeneration.

4. Claim 24 of the subject Application recites a method of inducing organized, functional bone formation at a site of bone infirmity in a human, comprising the steps of:

(a) transforming a cultured mesenchymal stem cell with a DNA encoding human bone morphogenesis protein 2 (BMP-2);

(b) culturing the cultured mesenchymal stem cell transformed in step (a), under conditions enabling expression of said DNA encoding bone morphogenesis protein 2; and

(c) implanting said cultured mesenchymal stem cell in an allogeneic subject, at a site of bone infirmity

whereby autocrine and paracrine effects of expressed human bone morphogenesis protein 2 at said site of bone infirmity result in organized, functional bone formation, thereby inducing organized, functional bone formation at a site of bone infirmity.

5. The specification provides exemplification of the claimed material, whereby mesenchymal stem cells transduced with a BMP-2 containing construct effectively stimulated functional bone formation, including formation specifically along defect edges. Example 1 demonstrated that regulated expression of BMP-2 was highly effective in promoting bone formation at a segmental defect site. Example 4 demonstrated that marrow osteoprogenitor cells genetically modified to express BMP were effective in promoting bone

formation as well. Example 8 demonstrated the advantage of combined paracrine and autocrine effects afforded by the use of the ex-vivo transformed cells, which resulted in superior bone formation, when compared to paracrine effects alone, and Example 11 demonstrated that only MSC expressing BMP-2 (MSC-BMP-2) provided was incorporated in newly formed bone trabecules, and formed superior quantitative and qualitative bone, this despite the fact BMP-2 was secreted at a roughly 100 times lower concentration than that of CHO cells transduced to express BMP-2 and 100 times lower concentration than the amount of BMP-2 loaded on collagen sponges.

6. The Examiner rejected the claims of the above-identified application as allegedly being obvious to one skilled in the art, based on Ahrens et al. (DNA and Cell Biology, Volume 12, NO. 10, pages 871-880, 1993) and in view of United States Patent No. 5,763,416 (Bonadio et al.) and United States Patent No. 6,048,964 (Lee et al.). As I understand, the Examiner alleged that Bonadio's described targeting of progenitor cells *in vivo*, combined with Ahren's described transduced cell render the claims obvious to one of ordinary skill in the art. The Examiner rejected claim 27 as allegedly being obvious to one skilled in the art in view of the above cited references, further in view of Wozney, and claim 28 as allegedly being obvious to one skilled in the art in view of the above cited references, further in view of Hattersley.

7. It is my opinion that the cited references do not render the invention obvious. In my opinion, Bonadio does not provide a credible foundation for a method of stimulating bone formation at a site of a bone infirmity by implanting a mesenchymal stem cell transformed/transduced with a BMP-2 construct. Bonadio targets a heterogeneous population of cells. While Bonadio describes specific targeting of progenitor cells, this contention is not credible, since stem cells if present, are in negligible amount, at the site of gene transfer. Cellular uptake of DNA is a complex process, and varies in terms of the different cell types and/or stage of differentiation of such cells, and/or the efficiency or even plausibility of such uptake. The type of vector employed will also necessarily affect the kinetics of such uptake.

8. Bonadio used a gene transfer system, which is not suitable for transfection of undifferentiated cells. For example, the adenoviral vectors used by Bonadio

depend upon CAR-mediated uptake, a receptor known to not readily be expressed on immature, noncommitted progenitor cells (see for example, Rebel V.I. et al., Stem Cells (2000) 18: 176-82; Zhao Q. et al., Blood (1994) 84:3660-6), at the time the invention was made. Therefore, based on my 18 years of experience and expertise in the field of Molecular and Cell Biology it is not credible, that at the time the invention was made, direct gene transfer experiments conducted by Bonadio targeted undifferentiated cells. Therefore, in my opinion Bonadio does not provide a foundation that BMP gene transfer supplies more than paracrine effects for healing a bone infirmity and Bonadio cannot predict the organized functional bone formation of the instant invention which occurs as a consequence of transfer of an enriched population of ex-vivo cultured BMP-2 expressing MSC (MSC-BMP-2).

9. The experiments in examples 7, 9, and 11 conducted with CHO cells expressing BMP-2, and collagen sponges loaded with purified BMP-2 can serve as an indication of the contrast between what Bonadio describes and the instant invention. Differentiated cells at a site of bone infirmity are the bulk recipients of the gene construct of Bonadio, or responders to the purified protein produced at the site, and not stem or progenitor cells. Such cells did not home to the site of bone infirmity, and did not produce as qualitatively or quantitatively organized functional bone, as compared to the MSC-BMP-2. The CHO-BMP and loaded sponge controls serve as reliable indicators as to what direct gene transfer produces at a fracture site.

10. Lee describes osteogenesis via local administration of a morphogenic protein. Osteogenesis is assumed in Lee, based solely on alkaline phosphatase production by osteoblasts in culture. Lee does not provide any demonstration of bone formation, but rather production of alkaline phosphatase alone, *in vitro*. There is no indication, based on Lee, that ex-vivo cultured BMP-2 transduced MSC promote organized functional bone formation *in vivo*. Moreover, Lee's findings rely solely on paracrine effects of the BMP for stimulating osteoblast AP production. In my opinion, the data presented in the subject Application demonstrate that paracrine effects alone do not result in organized, functional bone formation.

11. Ahrens discloses *in vitro* responses of progenitor cells to a group of osteoinductive compounds (which include, *inter-alia*, a BMP). Ahrens provides no foundation for the likelihood that implantation of such cells, transduced only with a vector expressing a BMP, *in vivo*, will stimulate organized, functional bone formation at a site of bone infirmity. Such a result is predicated on appropriate cell homing and orientation along the defect edges, a result, which could not have been foreseen, based on Ahrens.

12. Neither Ahrens nor Lee describe, or provide a foundation for cells alone, *in lieu* of any other osteoinductive matrix, stimulating bone formation. Certainly neither describe nor provide any foundation for organized, functional bone formation of the instant invention, i.e. bone formation along fracture defect edges, as demonstrated in the subject Application. Accordingly, the differences in implantation of an enriched MSC population expressing BMP-2 promoting organized bone formation, within the boundaries of the fracture edges, and lack of appreciable bone resorption could not have been predicted, based on Bonadio, Ahrens, or Lee, alone or in combination.

13. The Examiner cited the Fang reference in support of Bonadio's mechanism being the same as that of the claimed invention. In my opinion, this is incorrect. Fang does not and cannot support this position for the following reasons:

    - Fang describes BMP-4 uptake by fibroblasts;
    - Since the BMP-4 uptake is by fibroblasts, its effect on bone formation must necessarily be via a paracrine mechanism;
    - The claimed invention, however, is directed to use of an ex-vivo cultured BMP-2 transformed/transduced progenitor cell, which exerts both paracrine and autocrine effects. The subject Application demonstrated that paracrine effects alone are insufficient to promote organized bone formation. Thus, Bonadio, even in view of Fang, does not support or provide a foundation for the claimed invention.

14. Moreover, Fang in fact contradicts Bonadio's contention that the mechanism of gene transfer results in the specific targeting of progenitor cells, since in Fang,

the construct was expressed by fibroblasts. Thus, Fang questions the credibility of Bonadio as to the mechanism of action of direct gene transfer in inducing bone formation.

15. Paracrine effects of BMP-2 are not sufficient to promote organized bone formation and prevent bone resorption at the site of a bone infirmity. Example 11 specifically demonstrates that better bone formation occurs when BMP is expressed predominantly by the MSCs as this provides for autocrine and paracrine effects, which yields better, qualitative and quantitative bone formation, which is organized along the defect edges, and subject to no appreciable resorption.

16. Wozney describes expression of a BMP2 receptor. However, no description of the use of *ex-vivo* cultured MSC transduced/transformed with BMP-2, in inducing organized functional bone formation at a site of bone infirmity in cells responding to the growth factor is disclosed in Wozney, Bonadio, Ahrens, or Lee, alone or in combination. In my opinion, therefore, Wozney's findings, when combined with the other cited references, neither implies, nor renders obvious the paracrine and autocrine effects on bone formation by implantation of ex-vivo MSC transduced/transformed with BMP-2, further comprising a BMP-2 receptor.

17. Hattersley describes the use of PTH and its receptor in combination with a BMP, for applications in tissue repair. Hattersley neither alone, nor in combination with the above cited references describes, nor provides any foundation for the use of *ex-vivo* cultured MSC transduced/transformed with BMP-2, with or without further expression of PTH and its receptor in inducing organized functional bone formation at a site of bone infirmity.

18. It is my opinion that none of the cited references, alone or in combination, describe, or provide a foundation for inducing enhanced, organized, functional bone formation at a site of bone infirmity in a human by implanting in an allogeneic subject, an *ex-vivo* cultured MSC transduced/transformed with a human BMP-2. The Bonadio, Ahrens, and Lee disclosures do not credibly lead

one to a population of cells capable of forming organized, functional bone at a site of bone infirmity, since it is improbable that Bonadio targets the cultured population, and further combination with Lee or Ahrens *in vitro* results do not support bone formation, in particular in the absence of exogenous provision of an osteoinductive matrix or compound.

19. The combination of Wozney, Hattersley, Fang, Bonadio, Ahrens, and Lee could not possibly have predicted the unexpected results obtained in the claimed invention, which resulted in enhanced, organized, functional bone formation at a site of bone infirmity. *In-vivo* studies (*see Example 11*), demonstrated that engineered progenitor cells (C3H-BMP2), in comparison to administration of 3 μg recombinant human BMP2, or engineered non progenitor cells (CHO-BMP2) produced enhanced bone formation, and most surprisingly, that the formation was in alignment with the original defect edge, this despite the fact that greater amounts of BMP-2 were secreted from the CHO BMP-2 cells, or were loaded on the collagen sponges.

20. The paracrine effects of BMP-2, as described in Bonadio and Fang, are not sufficient to promote organized bone formation and prevent bone resorption at the site of a bone infirmity. Nor does the combination of Bonadio, Fang, Ahrens, Wozney, Hattersley, or Lee lead one to the unexpected finding that an enriched population of MSCs expressing BMP-2 are particularly useful in promoting organized functional bone, by a process mimicking that which occurs in spontaneously healing bones, and producing qualitatively and quantitatively better bone than that achieved with delivery of the BMP via paracrine effects alone.

21. In view of the reasons and the facts described above, one skilled in the art would not be able to predict the organized, functional bone induction at a site of bone infirmity produced via implantation of *ex- vivo* transformed/transduced MSCs with BMP-2, as claimed in the subject Application.

The undersigned further declares that all statements made herein of his own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: November 15, 2006

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